

**PATENT APPLICATION**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of

Docket No: Q83588

Shogo ISHIUCHI

Appln. No.: 10/509,379

Group Art Unit: 1612

Confirmation No.: 5052

Examiner: Snigdha MAEWALL

Filed: September 27, 2004

For: REMEDY FOR GLIOBLASTOMA

**DECLARATION UNDER 37 C.F.R. § 1.132**  
**(originally executed March 12, 2009; corrected for typographical errors)**

Mail Stop Amendment  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Shogo ISHIUCHI, hereby declare and state:

THAT I am a citizen of JAPAN;

THAT I have received the degree of M.D. in 1985 from Gunma University; and

THAT I have been employed by Department of Neurosurgery, Gunma University School of Medicine since 1999, where I hold a position as Associate Professor, with responsibility for Medical Director.

I further declare that I am the inventor of the above-identified application and familiar with the Office Action mailed November 21, 2008. In the Office Action, the Office asserts that claims 13-16 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for "7-acetyl-5-(4-aminophenyl)-8(R)-methyl-8,9-dihydro-7H-1,3-dioxolo [4,5-h][2,3]benzodiazepine (Talampanel) in treating glioblastoma, does not reasonably provide enablement for each and every compound claimed. Paragraph 4 on page 5 of the Office Action.

**Test 1 Inhibitory effects of compound B on glioblastoma growth -in vitro-**

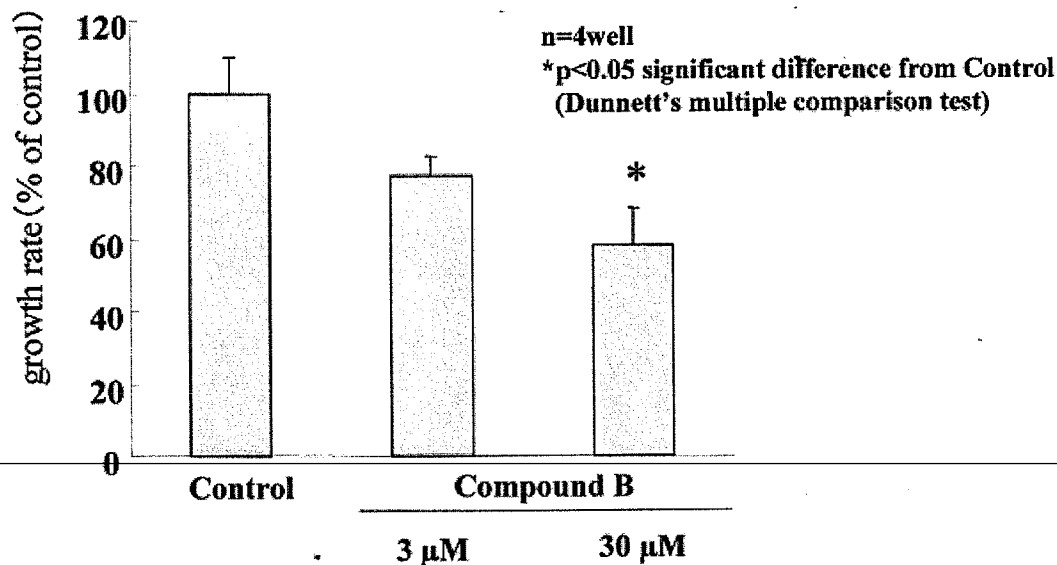
Human glioblastoma cell (CGNH-89 cell line) was used at this experiment. The cells were inoculated at  $1 \times 10^5$  cells per well into well plates containing Eagle's medium with 5% serum. These cells were randomly divided in the following three groups 1 day after seeding: control group, 3 $\mu$ M compound B applied group and 30 $\mu$ M compound B applied group. The cells were incubated for 96 hours. The CGNH-89 cell was cultured in DMEM (Dulbecco's modified Eagle's medium) with glutamine-free and glutamate-free. Each group was set at 4 wells.

The anti-tumor action was evaluated by determining cell count per well using a hemocytometer 96 hours after culturing.

The results are shown as mean  $\pm$  standard error and statistically analyzed by the Dunnett's multiple comparison test. Significance level was set at  $p < 0.05$ .

As shown in Figure 1, compound B inhibited dose-dependently the growth of CGNH-89 cells with 42.1% inhibition at 30 $\mu$ M.

**Figure 1**



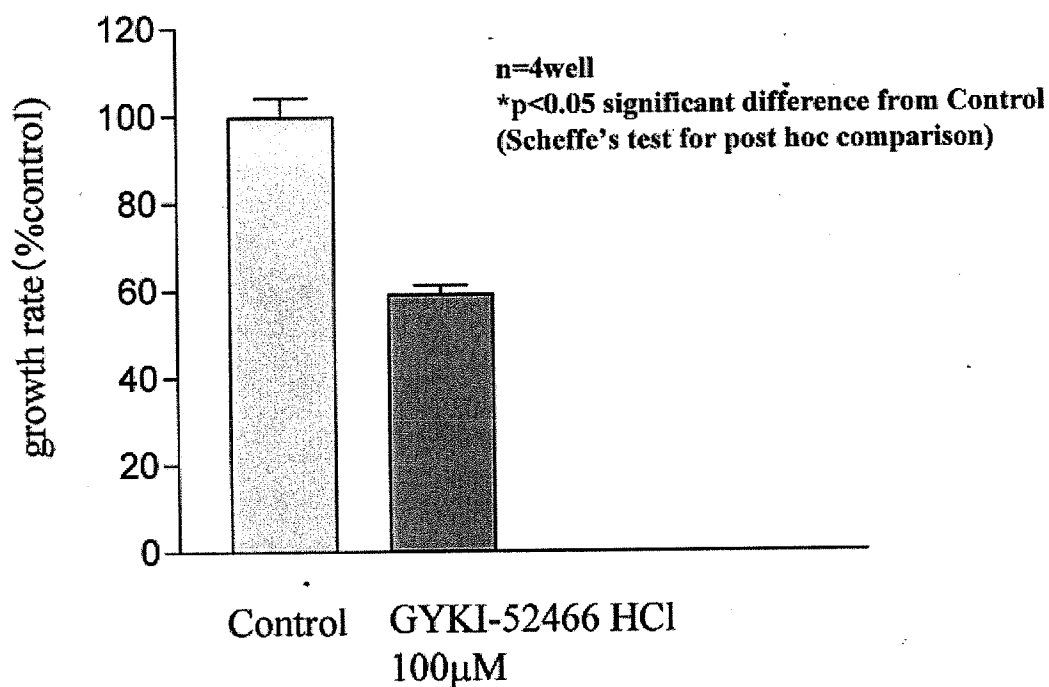
## Test 2 Inhibitory effects of GYKI52466 HCl salt on glioblastoma growth -in vitro-

Human glioblastoma cell (CGNH-89 cell line) was used at this experiment. The cells were inoculated at  $1 \times 10^5$  cells per well into well plates containing Eagle's medium with 5% serum. These cells were randomly divided in the following two groups 1 day after seeding: control group and 100 $\mu$ M GYKI52466 HCl salt applied group. The cells were incubated for 96 hours. The CGNH-89 cell was cultured in DMEM (Dulbecco's modified Eagle's medium) with glutamine-free and glutamate-free. Each group was set at 4 wells. The anti-tumor action was evaluated by determining cell count per well using a hemocytometer 96 hours after culturing.

The results are shown as mean  $\pm$  standard error and statistically analyzed by the Scheffe's test for post hoc comparison. Significance level was set at  $p < 0.05$ .

As shown in Figure 2, GYKI52466 HCl salt inhibited the growth of CGNH-89 cells with 41% inhibition at 100 $\mu$ M ( $p < 0.001$ ).

Figure 2



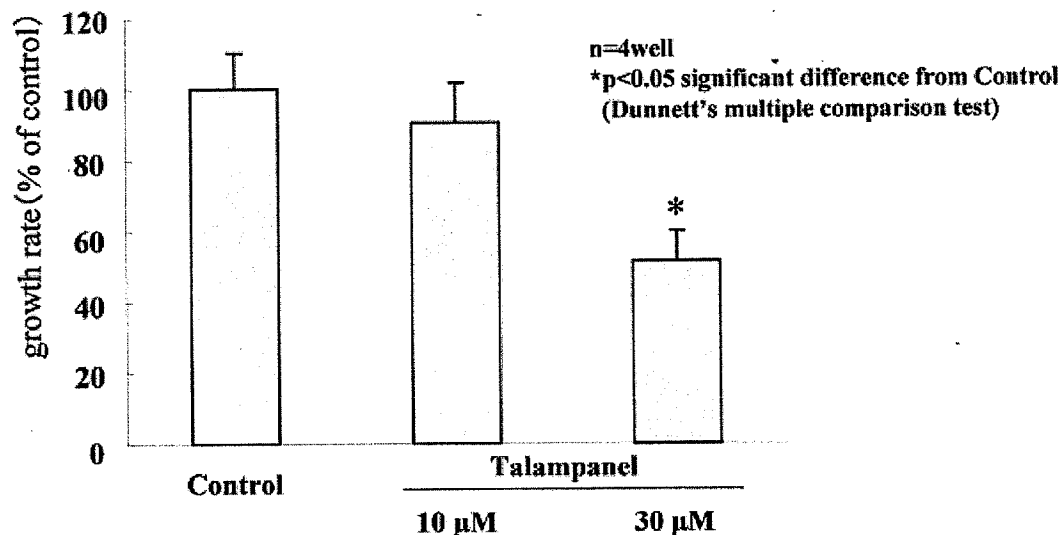
**Test 3 Inhibitory effects of Talampanel on glioblastoma growth -in vitro-**

Human glioblastoma cell (CGNH-89 cell line) was used at this experiment. The cells were inoculated at  $1 \times 10^5$  cells per well into well plates containing Eagle's medium with 5% serum. These cells were randomly divided in the following three groups 1 day after seeding: control group, 10 $\mu$ M Talampanel applied group and 30 $\mu$ M Talampanel applied group. The cells were incubated for 96 hours. The CGNH-89 cell was cultured in DMEM (Dulbecco's modified Eagle's medium) with glutamine-free and glutamate-free. Each group was set at 4 wells.

The anti-tumor action was evaluated by determining cell count per well using a hemocytometer 96 hours after culturing.

The results are shown as mean  $\pm$  standard error and statistically analyzed by the Dunnett's multiple comparison test. Significance level was set at  $p < 0.05$ .

As shown in Figure 3, Talampanel inhibited dose-dependently the growth of CGNH-89 cells with 48.7% inhibition at 30 $\mu$ M.

**Figure 3**

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: September 3, 2009

  
Shogo ISHIUCHI